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BRIEF COMMUNICATIONS

Lewy bodies in grafted neurons in subjects with Parkinson's disease suggest host-to-graft disease propagation

Jia-Yi Li[1], Elisabet Englund[2], Janice L Holton[3], Denis Soulet[1], Peter Hagell[4], Andrew J Lees[3], Tammaryn Lashley[3], Niall P Quinn[5], Stig Rehncrona[6], Anders Björklund[7], Håkan Widner[4], Tamas Revesz[3,9], Olle Lindvall[4,8,9] & Patrik Brundin[1,9]

Two subjects with Parkinson's disease who had long-term survival of transplanted fetal mesencephalic dopaminergic neurons (11–16 years) developed α-synuclein–positive Lewy bodies in grafted neurons. Our observation has key implications for understanding Parkinson's pathogenesis by providing the first evidence, to our knowledge, that the disease can propagate from host to graft cells. However, available data suggest that the majority of grafted cells are functionally unimpaired after a decade, and recipients can still experience long-term symptomatic relief.

Two sham surgery–controlled trials of neural transplantation in Parkinson's disease did not reach their primary endpoints[1,2]. However, previous open-label trials with grafts of fetal ventral mesencephalic tissue reported long-lasting functional benefits in subjects with Parkinson's disease[3]. Positron emission tomography (PET) studies showed that grafted neurons were functionally integrated and released dopamine for more than 10 years after surgery[4]. In postmortem neuropathological studies performed 3–4 years after transplantation, large numbers of dopaminergic neurons were found in the grafts[1,2,5].

We now report that grafted cells can survive up to at least 16 years in subjects with Parkinson's disease. Large numbers of transplanted dopaminergic neurons were found in two subjects who had undergone bilateral implantation of fetal mesencephalic tissue into the putamen (subject 3 in the Lund series; left graft 16 years before death, right graft 12 years before death) or both putamen and caudate nucleus (subject 8; left graft 13 years before death, right graft 11 years before death)[6]. Graft survival was confirmed by clinical improvement at 5 months up to at least 3 years after surgery in subject 3 (ref. 6).

Both subjects died from causes unrelated to grafting (**Supplementary Methods** online). In their substantiae nigrae, the subjects had histopathological changes characteristic of Parkinson's disease: severe

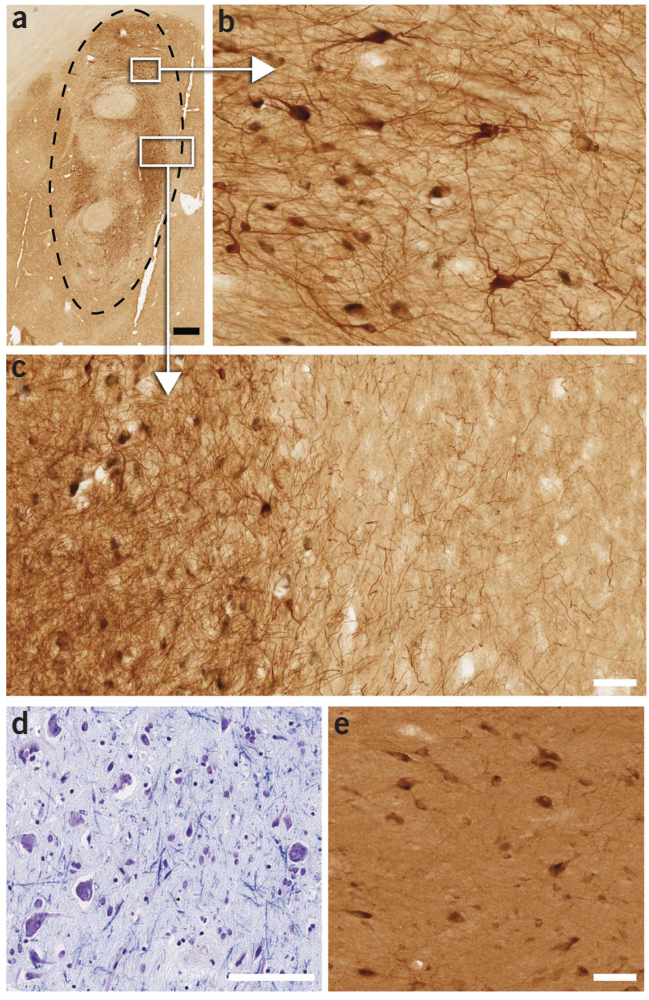

Figure 1 Surviving dopaminergic neurons labeled with antibody to tyrosine hydroxylase in a graft transplanted 16 years before death in subject 3. (**a**–**c**) Surviving cells mainly exist in the periphery of the graft (**a**) with classical morphology of dopaminergic neurons (**b**), extending long processes into the host striatum (**c**). (**d**) Luxol fast blue staining shows good morphology of grafted neurons and myelinated axons. (**e**) Girk2-positive dopaminergic neurons are shown from a graft. Scale bars, 500 μm in **a**; 100 μm in **b**–**e**.

[1]Neuronal Survival Unit, Wallenberg Neuroscience Center, Department of Experimental Medical Science, 221 84 Lund, Sweden. [2]Division of Neuropathology, Lund University Hospital, 221 85 Lund, Sweden. [3]Queen Square Brain Bank for Neurological Disorders, Department of Molecular Neuroscience, UCL Institute of Neurology, University College London, Queen Square, London, WC1N 3BG, UK. [4]Division of Neurology, Lund University Hospital, 221 85 Lund, Sweden. [5]Sobell Department of Motor Neuroscience and Movement Disorders, UCL Institute of Neurology, University College London, Queen Square, London, WC1N 3BG, UK. [6]Division of Neurosurgery, Lund University Hospital, 221 85 Lund, Sweden. [7]Neurobiology Unit and [8]Section of Restorative Neurology, Wallenberg Neuroscience Center, 221 84 Lund, Sweden. [9]These authors are senior authors. Correspondence should be addressed to P.B. (patrik.brundin@med.lu.se).

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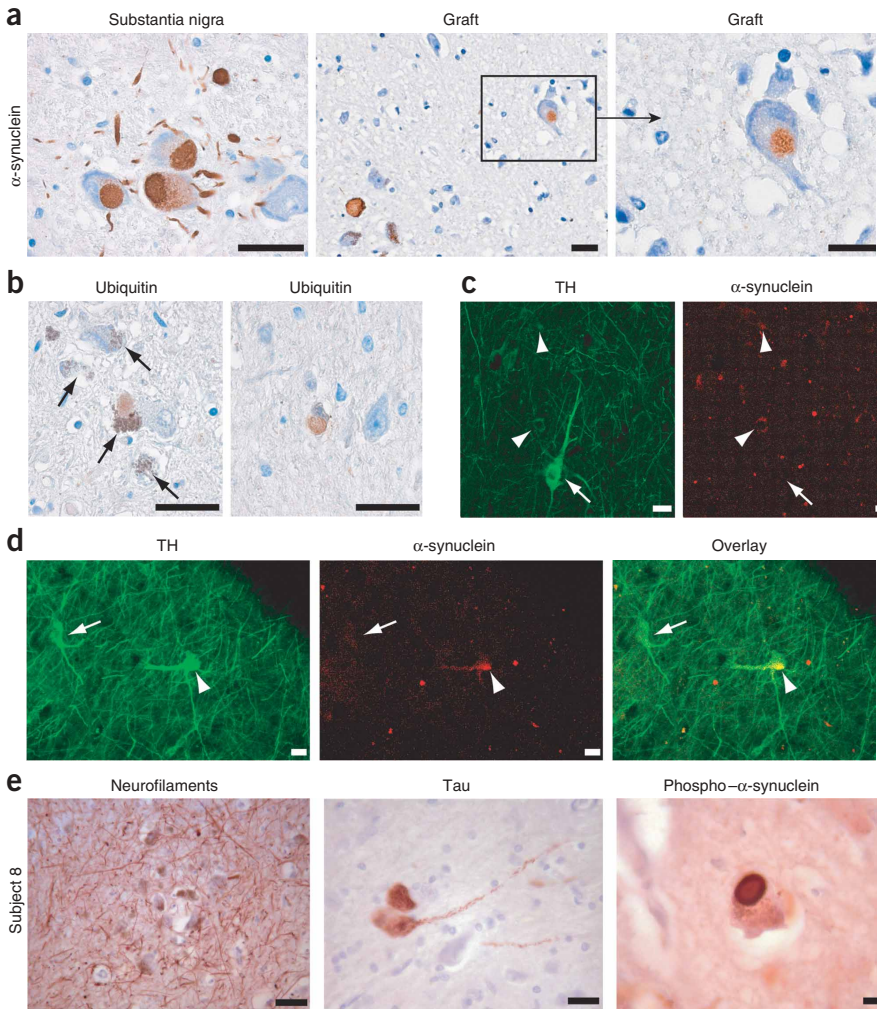

Figure 2 α-synuclein–positive Lewy bodies in host substantia nigra and grafted dopaminergic neurons in subject 3 (**a**–**d**) and subject 8 (**e**). (**a**) Classical Lewy bodies and Lewy neurites in neurons of the substantia nigra (left) and in the grafts (middle and right) are immunoreactive for α-synuclein in paraffin-embedded tissue sections. (**b**) Lewy bodies in the grafts are immunoreactive for ubiquitin in paraffin-embedded tissue sections. Arrows (left) point to dopaminergic neurons containing a large number of pigmented granules. (**c**) Double immunolabeling shows colocalization of tyrosine hydroxylase (TH, green) and α-synuclein (red) in some dopaminergic neurons of the substantia nigra (arrowheads). One dopaminergic neuron with good morphology (arrows) does not contain detectable α-synuclein. (**d**) Double immunolabeling shows colocalization of tyrosine hydroxylase (green) and α-synuclein (red) in a graft (arrowheads). One dopaminergic neuron (arrows) does not contain detectable α-synuclein. (**e**) Histological sections of grafts from subject 8 stained by immunohistochemistry for neurofilament (left), human tau (middle) and phosphorylated S129 on α-synuclein (right). Neurofilament staining shows numerous axons and neurons containing pigmented granules in a graft (left). Neurons with punctate tau positivity are suggestive of pretangles (middle). A typical Lewy body stained with an antibody to phospho–α-synuclein is shown in a grafted neuron (right). Scale bars, 40 μm, except in **e** (middle and right), 15 μm. Grafting procedures in both subjects were performed with informed consent. Informed consent was also obtained for postmortem investigation, including for the use of brain tissue for research in both cases. Grafting and all procedures related to postmortem tissue handling were approved by the Regional Ethical Review Board in Lund, Sweden and by the Joint Research Ethics Committee of the National Hospital for Neurology and Neurosurgery and UCL Institute of Neurology, UK. Brain donations to the Queen Square Brain Bank for Neurological Disorders are approved by a London Multi-Centre Research Ethics Committee.

loss of pigmented neurons and α-synuclein– and ubiquitin-positive Lewy bodies and Lewy neurites in surviving neurons. Cortical regions showed diffuse cortical Lewy body–type pathology in both subjects. Numerous tyrosine hydroxylase–immunoreactive, presumed dopaminergic neurons were found primarily at the periphery of the transplants in subject 3 (**Fig. 1a–c**). In both subjects, the majority of dopaminergic neurons had long processes, forming dense networks in the grafts and surrounding striatum (**Fig. 1**). Many neurons contained neuromelanin and possessed myelinated axons (**Fig. 1b** and **Fig. 2a**,**b**,**e**). The tyrosine hydroxylase–immunoreactive neurons, grafted at different time points, were similar morphologically (**Supplementary Fig. 1** online). Some grafted neurons were immunopositive for Girk2, a marker of dopaminergic neurons in substantia nigra pars compacta (**Fig. 1e** and **Supplementary Fig. 2** online), whereas others expressed calbindin, a marker of ventral tegmental area and substantia nigra dorsal tier pars compacta neurons (**Supplementary Fig. 2**). Iba1-positive microglia accumulated around the grafts, but CD68 immunohistochemistry did not indicate strong activation (**Supplementary Fig. 3** online). Quantitative analysis in subject 3 showed 12,100–29,500 tyrosine hydroxylase–immunoreactive neurons per injection track on the first grafted side and 14,400–27,600 tyrosine hydroxylase–immunoreactive cells per injection track on the second side, indicating that the numbers of long-surviving (12–16 years) dopaminergic neurons were of the same magnitude as in subjects surviving 18 months to 4 years after transplantation[5,7].

In both cases, and similarly to surviving neurons in the substantia nigra pars compacta, grafted neurons, some of which were clearly pigmented, contained characteristic α-synuclein– and ubiquitin-positive Lewy bodies and Lewy neurites (**Fig. 2a**,**b** and **Supplementary Fig. 4** online), which were also labeled by an antibody recognizing α-synuclein phosphorylated at Ser129 (**Fig. 2**). A small number of grafted neurons showed punctate cytoplasmic tau reactivity (pretangle) with phospho-tau immunohistochemistry (**Fig. 2e**).

We found that 40% of tyrosine hydroxylase–positive cells contained detectable amounts of α-synuclein in the 12-year-old implants, as did 80% of the tyrosine hydroxylase–positive cells in the 16-year-old grafts in subject 3. Dopaminergic neurons in the host midbrain had similar expression of α-synuclein (**Fig. 2c**,**d**). The difference in α-synuclein expression between the grafts in subject 3 supports the notion that increased intracellular α-synuclein is time- or age-dependent, which is consistent with the fact that age could be a risk factor for Parkinson's disease[8].

The most striking finding in both cases was the presence of Lewy bodies and Lewy neurites in the long-surviving grafted dopaminergic

neurons that were morphologically indistinguishable from those seen in the substantia nigra pars compacta neurons in Parkinson's disease. The Lewy bodies in grafted cells were α-synuclein and ubiquitin immunoreactive and were also stained with an antibody recognizing α-synuclein phosphorylated at Ser129, strongly suggesting the presence of disease-related, post-translationally modified and aggregated α-synuclein[9].

The mechanisms leading to the initiation and spread of α-synuclein pathology in Parkinson's disease are not well understood, and those underlying similar pathological changes in grafts are also obscure. Because the dopaminergic neurons were grafted into an ectopic site, they may have been exposed to an unfavorable microenvironment. Studies in rodents have shown that grafted dopaminergic neurons can receive corticostriatal afferents[10], and glutamatergic excitotoxic damage might have occurred in the grafts. Exposure to amphetamine or the toxins MPTP and rotenone can lead to an unfavorable microenvironment and upregulation of α-synuclein in nigral neurons, promoting its aggregation[11]. Moreover, it has been suggested that Parkinson's disease is coupled to impaired neurotrophic support[12], and grafts may lack appropriate trophic signaling in the striatum in Parkinson's disease. Finally, microglial activation occurs in the basal ganglia in Parkinson's disease and, combined with the low-level inflammation indicated by graft-related microglia (**Supplementary Fig. 3**), could contribute to α-synuclein aggregation in grafted cells[13].

Stereotypic topographical progression of Lewy body pathology has been suggested to be a characteristic feature in Parkinson's disease[14]. On the basis of experimental data, including those showing the acceleration of amyloid deposition after exogenous seeding[15], the hypothesis of 'permissive templating' has been proposed to explain disease propagation along neuronal pathways in neurodegenerative diseases, including Parkinson's disease[16]. Our observations may provide support for such a hypothesis, implicating a 'prion-like' mechanism. One could, therefore, speculate that the α-synuclein aggregation and deposition observed in the transplanted dopaminergic neurons was triggered by misfolded α-synuclein in the host, which was transmitted into grafted cells.

Increased α-synuclein abundance and the formation of Lewy bodies could be detrimental to grafted dopaminergic neurons and potentially limit the duration of efficacy of cell replacement therapy. Indeed, an inverse correlation has been shown between levels of α-synuclein and tyrosine hydroxylase in substantia nigra dopaminergic neurons[8]. F-dopa and raclopride PET imaging, however, shows that mesencephalic grafts can still synthesize and release normal amounts of dopamine 10 years after transplantation[4]. Therefore, even though some grafted dopaminergic neurons undergo pathological changes similar to those found in Parkinson's disease, the majority do not seem functionally impaired, and recipients may still experience long-term symptomatic relief after a decade.

Note: Supplementary information is available on the Nature Medicine website.

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AUTHOR CONTRIBUTIONS

J.-Y.L. designed and performed the detailed morphological analysis of most of the material from subject 3. E.E. and J.L.H. did autopsy and routine neuropathology of subjects 3 and 8, respectively. J.L.H. and T.R. designed and T.L. performed the detailed morphological analysis of subject 8. A.B. provided expertise in neural transplantation. D.S. provided expertise regarding graft reconstruction, imaging and microglia staining and also generated figures. P.H. assisted in the care of subject 3 and provided data related to clinical follow-up. H.W. and N.P.Q. took care of subjects 3 and 8, respectively. A.J.L. provided clinical evaluation for subject 8. S.R. operated on both subjects. P.B. dissected and prepared tissue for both surgeries and participated in the morphological assessment of subject 3. O.L. took care of subject 3 and headed the clinical transplantation program. J.-Y.L., J.L.H., T.R., O.L. and P.B. wrote the manuscript. All authors gave input to the manuscript.

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1. Olanow, C.W. *et al. Ann. Neurol.* **54**, 403–414 (2003).
2. Freed, C.R. *et al. N. Engl. J. Med.* **344**, 710–719 (2001).
3. Björklund, A. *et al. Lancet Neurol.* **2**, 437–445 (2003).
4. Piccini, P. *et al. Nat. Neurosci.* **2**, 1137–1140 (1999).
5. Mendez, I. *et al. Brain* **128**, 1498–1510 (2005).
6. Hagell, P. *et al. Brain* **122**, 1121–1132 (1999).
7. Kordower, J.H. & Sortwell, C.E. *Prog. Brain Res.* **127**, 333–344 (2000).
8. Chu, Y. & Kordower, J.H. *Neurobiol. Dis.* **25**, 134–149 (2007).
9. Anderson, J.P. *et al. J. Biol. Chem.* **281**, 29739–29752 (2006).
10. Doucet, G. *et al. Exp. Neurol.* **106**, 1–19 (1989).
11. Vila, M. *et al. J. Neurochem.* **74**, 721–729 (2000).
12. Mogi, M. *et al. Neurosci. Lett.* **270**, 45–48 (1999).
13. Sigurdsson, E.M., Wisniewski, T. & Frangione, B. *Trends Mol. Med.* **8**, 411–413 (2002).
14. Braak, H. *et al. Neurobiol. Aging* **24**, 197–211 (2003).
15. Meyer-Luehmann, M. *et al. Science* **313**, 1781–1784 (2006).
16. Hardy, J. *Biochem. Soc. Trans.* **33**, 578–581 (2005).